Nucleic Acid Detection

Detection of Nucleic Acids Using a Novel Enhanced Luminol System

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Introduction

Many nonisotopic methods have been developed for nucleic acid detection, and recently some of these methods have been improved to the point where their sensitivity equals that of their isotopic counter-parts. However, the robustness of these sensitive methods, usually based on alkaline phosphatase detection with 1,2-dioxetane substrates, tends to suffer from a high degree of signal-to-noise variability. Aside from this lack of robustness of the current methods, another drawback is the inordinate amount of processing time involved in the procedure and the length of exposure time needed to obtain the desired sensitivity. These disadvantages undoubtedly contribute to the lack of wide acceptance of nonisotopic nucleic acid detection methods. We recently developed a complete system for the chemiluminescent detection of nucleic acids in Northern and Southern blotting applications. This system combines a novel enhanced luminol substrate for horseradish peroxidase (HRP) with optimized hybridization and blocking steps that ensure consistent results with sensitivity equivalent to 32P. This robust system also has the advantages of greatly reduced processing and film exposure times. Post-hybridization processing time has been reduced from the standard 2.5 hours to 1 hour. Film exposure times range from 0.5-10 minutes with the substrate emitting light with relatively constant intensity over a 6-hour period, thus allowing for multiple exposures.

Materials and Methods

Equipment

The UV Stratalinker® is from Stratagene (San Diego, CA).

Reagents

Film developing chemicals were purchased from Sigma Chemical (St. Louis, MO). Biotinylated and 32 P-labeled cRNA probes for the S. cerevisiae TCM1 and INO2 genes were synthesized using either the North2South™ in vitro Transcription Kit (Pierce) or the MAXIscript™ System from Ambion (Austin, TX) and [a-32 P] UTP from Amersham Life Sciences (Arlington Heights, IL) from linearized plasmid pAB309D (TCM1) or pGEM-INO2 (INO2). DIG-labeled cRNA probes were synthesized using the DIG RNA Labeling System from Boehringer Mannheim (Indianapolis, IN). Biotinylated DNA probes were synthesized using the North2South™ DNA Labeling Kit from Pierce and a restriction fragment containing the gene encoding green fluorescent protein (GFP).

DIG-labeled probes were detected using the DIG High Prime Labeling and Detection Kit with CSPD® from Boehringer Mannheim. All other equipment and products were from Pierce.

Northern Blot
Total yeast RNA was isolated and fractionated on a formaldehyde/agarose gel and transferred to Biodyne® B Nylon Membrane as described previously.1 DIG-labeled cRNA probes were hybridized and detected according to the manufacturer's specifications. Radiolabeled cRNA probes (108 cpm/µg) were hybridized and detected as described previously.1 Biotinylated cRNA probes were hybridized and detected using the North2South™ Chemiluminescent Detection System as follows. Following capillary transfer, nucleic acids were UV-cross-linked to the membrane. The membranes were then pre-hybridized in hybridization buffer for at least 30 minutes at 65°C. Following pre-hybridization, cRNA probe was added to the hybridization buffer to a final concentration of 5 ng/mL and the membranes were hybridized overnight at 65°C. The following day the membranes were washed 3 x 20 minutes at 65°C in stringency wash buffer. The membranes were then transferred to a plastic tray and blocked for 15 minutes in blocking buffer. Streptavidin-HRP conjugate was then added to the blocking buffer to a final concentration of 33 ng/mL and the membranes were incubated for 15 minutes at room temperature (RT) with gentle agitation. The membranes were then washed 4 x 5 minutes in wash buffer at RT with gentle agitation. Following washing, the membranes were incubated for 5 minutes in substrate equilibration buffer at RT. North2South™ Chemiluminescent Substrate Working Solution was prepared according to the manufacturer's recommendations and added to the membranes for 5 minutes. Membranes were exposed to film for various times as needed.

Southern Blot
Genomic DNA from S. cerevisiae was isolated as described previously.2 Genomic DNA was digested overnight with Hind III, EcoRI or SalI and Southern blots were carried out as described previously.3 Biotinylated cRNA probes for the INO2 gene were hybridized and detected using the North2South™ Chemiluminescent Detection System (as described previously, except hybridizations and washes were carried out at 55°C and probe was added to a final concentration of 30 ng/mL).

Plaque Lifts
Plaque lifts were carried out as described previously.3 Biotinylated DNA probes for the gene encoding GFP were hybridized and detected using the North2South™ Chemiluminescent Detection System as described for Southern blots.

Results and Discussion
The North2South™ Chemiluminescent Detection System was developed to address the current problems with chemiluminescent detection of nucleic acids.

While other commercially available systems now afford sensitivity equal to 32P-labeled probes, they suffer from long processing times and typically display problematic background, most often manifested in experiment-to-experiment varia-bility of signal-to-noise ratios. The North2South™ Chemiluminescent Detection System offers short process time, sensitivity equal to 32P, and extremely low background with minimal to no variability from experiment to experiment.

To assess the sensitivity of various detection protocols, we compared Northern blots of yeast total RNA using the North2South™ Chemiluminescent Detection System, the DIG labeling and detection system and radioactive labeling using 32P. Blots of a dilution series of total RNA starting from 5 µg and serially diluting down to 0.156 µg were hybridized with labeled TCM1 probe, detected according to the various protocols and exposed to film various times.

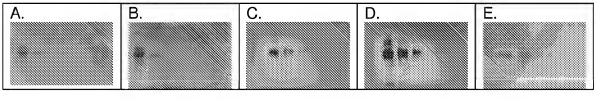

Figure 1. A Northern blot of serial dilutions of total yeast RNA (5 µg, 2.5 µg, 0.625 µg, 0.313 µg and 0.156 µg) was performed as described to measure TCM1 gene expression. In (A) and (B), a DIG-labeled TCM1 cRNA probe was hybridized and detected using the DIG labeling and detection system. In (C) and (D), a biotinylated TCM1 cRNA probe was hybridized and detected using the North2South™ Chemiluminescent Detection System. In (E) a 32P-labeled TCM1 cRNA probe was hybridized and detected. Film was exposed for: (A) 5 minutes, (B) 25 minutes, (C) 5 minutes, (D) 25 minutes and (E) 72 hours without intensifying screen.

As shown in Figure 1, the North2South™ Chemiluminescent Detection System offers greater sensitivity than the DIG-labeled probe and the 32P-labeled probe in a fraction of the time.

In addition to a high level of sensitivity, the North2South™ Chemiluminescent Detection System requires only a small amount of post-stringency wash process time. The optimized protocol requires only 1 hour of additional process time after the stringency washes, while the DIG detection system requires, in practice, almost 2.5 hours and far longer exposure times on film.

Although North2South™ Chemiluminescent Substrate has an extremely high light output, the North2South™ Chemiluminescent Detection System also offers extremely low background. In Figure 2, a Northern blot of yeast total RNA was probed with a biotinylated TCM1 cRNA probe exposed to film initially for (A) 5 seconds, (B) 1 minute, (C) 5 minutes and (D) overnight.

The strong signal and lack of background demonstrates an additional benefit of the North2South™ Chemiluminescent Detection System.

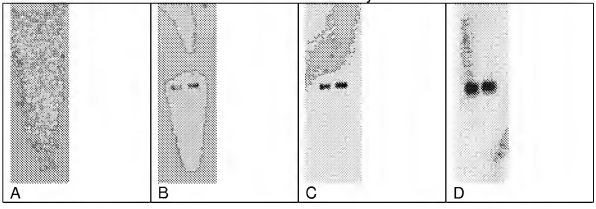

Figure 2. Northern blot demonstrating low background of the North2South™ Chemiluminescent Detection System. A Northern blot using 5 µg of yeast total RNA from different RNA preparations was performed as described. A biotinylated TCM1 cRNA probe was then hybridized and detected as described previously. Film exposures shown were: (A) 5 seconds, (B) 1 minute, (C) 5 minutes and (D) overnight.

Many researchers also need to perform Southern blot analysis. The North2South™ Chemiluminescent Detection System provides high sensitivity and extremely low background for this type of experiment. In Figure 3, a Southern blot of yeast genomic DNA was hybridized with a biotinylated cRNA probe for the single copy yeast INO2 gene. As shown in Figure 3, the North2South™ Chemiluminescent Detection System also provides high sensitivity and low background for Southern blotting applications.

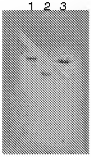

Figure 3. Southern blot detecting a single copy gene (INO2). A Southern blot using 5 µg of yeast genomic DNA was performed as described. A biotinylated INO2 cRNA probe was hybridized and detected as described previously. Lane 1: Hind III digest, Lane 2: EcoRI digest and Lane 3: SalI digest. Film was exposed for 1 minute. Note: The two bands seen in Lane 2 are due to the presence of an EcoRI site within the INO2 gene.

The need to carry out plaque lifts with low background often represents a daunting challenge for researchers even when using 32P labeling and detection strategies. We tested the North2South™ Chemiluminescent Detection System for its usefulness in plaque lift procedures. Phages were plated out with bacteria and incubated at 37°C to allow the plaques to form. Phage DNA was then transferred to nylon membrane, probed with a biotinylated DNA probe and detected using the North2South™ Chemiluminescent Detection System. As shown in Figure 4, the low to nonexistent background afforded by the North2South™ Chemiluminescent Detection System allows for distinct plaques to be picked out even in areas of high signal density.

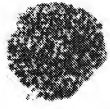

Figure 4. Plaque lifts were performed as described previously. A biotinylated DNA probe for the gene encoding GFP was hybridized and detected using the North2South™ Chemiluminescent Detection System. Film was exposed for 1 minute.

The North2South™ Chemiluminescent Detection System provides a high level of sensitivity with extremely low background for Northern blots, Southern blots and plaque lifts. In addition, this system has a short process time and a high level of light output, which allows for very short exposure times on film.

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